The British Veterinary Journal

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NOTICE TO CONTRIBUTORS

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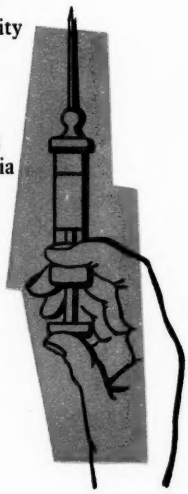

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Vol. 114 **May, 1958** **No. 5**

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EDITORIAL

PLANT POISONING RISKS

In this world there are so many things of vegetable and mineral origin which are poisonous that one is apt to wonder how man and his beasts have managed to survive all the risks which surround them.

If we leave the mineral poisons aside and think only in terms of the toxic substances which abide within the plant life of any country the toll of potential danger is very great.

In the springtime when nature is awakening again, any botanist who took a short walk around the hedges, ditches, and copses of a reasonably large farm could come back with his arms full of specimens which, to a greater or lesser degree, might produce toxic effects upon animals or man. How do the potential victims escape? Is it because many of the plants are distasteful and irritant to the palate or because there is an ample supply of other sweeter and more wholesome food. Even in those cases in which the poisonous product is attractive and delectable the experiences of the past and the memory of tragedies when man's only guide was that of trial and error, has delegated them to the list of things which are taboo. In this manner many plants are known to be poisonous to a dangerous degree, others come under strong suspicion and there are some which have been accused in error. With regard to animals, that indefinite attribute familiarly called instinct and which is probably born of bitter experience may be a safeguard also.

Fortunately in the case of animals, most of the plants that have toxic properties have to be ingested in fair quantity before poisonous effects can be observed. When consumed in subtoxic amounts the plants may do good and act as a digestive stimulant as does a sauce or an aperitif in the case of man. This is exemplified in modern ley farming procedures. Under the urge of economic pressure and following the advice of those who specialise in "grassland research", farmers

XUM

attempt to produce " two blades " where one grew before. They plant in the fields and meadows only those grasses which will grow in good abundance and, by the standards of scientific tests, contain those qualities which are wholesome and nutritious and are calculated to give the best returns in the growth and output of the animal body. The conception is good and most certainly the volume of the crops substantiates the scientists' ideas, but what do we find in practice? Animals, like men, do not always appreciate the foods which are said to be good for them. They become bored of that which is good for them and in search of change and tasteful stimulants they go to the hedgerows, the haunt of suspicious green things, and, with a perverseness which must be respected, imbibe a little " mustard and salt ". This being so and it being recognised that life must not be all dull and monotonous and a " little of what one fancies does a lot of good ", wise ley farmers leave the strips of the headlands to flourish according to nature's own design.

The presence of poisonous plants on a farm is an ever-present danger to the stock owner. His animals may leave them alone for years and then, for some reason or other, show an inexplicable desire to eat them and with serious results. Some plants appear to be dangerous at one time and not at another. The most obvious remedy for the danger would be to eliminate all the plants. Another method is to ascertain the nature and quality of the dangerous substance and prepare a suitable antidote which could be used in an emergency.

Elimination is not always a practical project in the case of those plants which grow rapidly and in great profusion. Bracken comes into this category. Cases of bracken poisoning occur every year with regular sequence and as it is not possible to expunge the plant completely from the countryside we hope that research workers will discover an antidotal treatment which can give a fair measure of protection should an emergency arise.

A CASE OF MALIGNANT TUMOUR IN THE DOG TREATED WITH SPECIFIC ANTIGEN

By A. V. CLARKE, B.Sc., M.R.C.V.S., and D. E. WATKINSON, M.R.C.V.S.
Crewkerne

Subject

ALASTIAN bitch aged 6½ years.

History

The bitch had been in good health until mid-December 1956 when she suffered from an attack of acute cystitis associated with a small cystic calculus. The calculus was passed and the cystitis resolved. The animal was presented again on 31st January, 1957. The owner said that in the past three weeks a sore patch had developed on the left fore foot and it had increased in size rapidly.

Signs

The area of the second phalanx was enlarged and thickened and there was an oval concave ulcerated area on the dorsal surface. The ulcerated area was bright pink in colour, moist and shining, and it had a pale whitish margin. It extended from the base of the nail to the junction with the other digits. There was a band of thickened tissue proximal to the ulcer.

Diagnosis

Clinically the lesion appeared to be a typical epithelioma. No biopsy was made for the infiltration was so extensive that it was considered impossible to make a biopsy without removing the whole of the affected toe. The owner would not consent to the latter measure.

Prognosis

The owner was advised that the only treatment we could suggest was early amputation of the affected limb and a grave prognosis was given because of the possibility that metastasis had occurred. The owner did not wish the dog to be disfigured by amputation. He asked for our permission to consult Dr. W. M. Crofton, with whom he was acquainted, and for our co-operation should the latter consider that the case was one which would respond to his method of treatment. To this we agreed.

Treatment

Dr. Crofton took swabs from the ulcerated surface of the lesion and later he reported that he had isolated a Gram-positive coccus. He prepared an autogenous vaccine and a series of injections of increasing strength. Treatment was commenced on 21st January, 1958. By this time the ulcerated area had increased in size and measured 30 mm. wide and 33 mm. long. The dog was very lame. At first the injections were given at weekly intervals but after one month, in the absence of any general systemic reaction, they were increased to two injections per week. This was continued until 23rd August, 1957, when the last injection was given. The dose of vaccine was progressively graduated from one-quarter million to 1,200 million.

Progress of Case

From the commencement of treatment no enlargement in the size of the ulcerated area was observed, though at first the thickening of the proximal side appeared to increase. Eight weeks after the first injection a thin layer of new skin was noted. At first this was white but later it became pigmented and it grew from the left side of the lesion. This process progressed slowly until the whole of the ulcerated surface was covered by new skin. This skin has now been covered by hair. Much of the original swelling has gone but some remains and, no doubt, the scar will be permanent. At the present time the dog is sound on this leg and in good health. She has been examined weekly since the termination of treatment and when last seen on 24th November, 1957, no recurrence had occurred, nor was there any sign of a secondary growth.

Discussion

We appreciate this is but an isolated case. We present it because in our experience we are convinced that this was a malignant tumour. Normally, a biopsy would have been performed but it was our considered opinion that a biopsy could not be justified without the removal of the whole of the affected toe.

The autogenous antigen (vaccine) was the only treatment adopted in this case and we are of the opinion that it was responsible for the resolution.

Summary

A tumour which exhibited clinical signs of malignancy was observed on the digit of a dog. The treatment of this tumour by the use of autogenous vaccine is described. No other treatment was applied. The lesion was resolved.

Acknowledgements

We thank W. B. Martin, M.R.C.V.S., who encouraged us to try this method of treatment, and Dr. W. M. Crofton, M.D., for his interest in the case and supplying the vaccine.

PROBLEMS OF SLAUGHER AND MEAT INSPECTION AND THE NEED FOR THEIR INVESTIGATION BY THE RESEARCH WORKER

PART I

By H. THORNTON, B.V.Sc., M.R.C.V.S., D.V.H., F.R.S.H.

Chief Veterinary Officer, City and County of Newcastle upon Tyne

(This article formed the subject matter of the Benjamin Ward Richardson Lecture delivered in London on the 11th December, 1957, under the ægis of the Royal Society of Health and published in the Royal Society of Health Journal for April, 1958.)

THE statement is frequently made that the inspector in the abattoir can provide valuable information regarding the incidence of pathological conditions and diseases

PLATE I

FIG. I
The growth before treatment.

FIG. II
After treatment.

(*Article by A. V. Clarke, on page 163*)

XUM

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(Article by A. V. Clarke, on page 163)

PLATE II

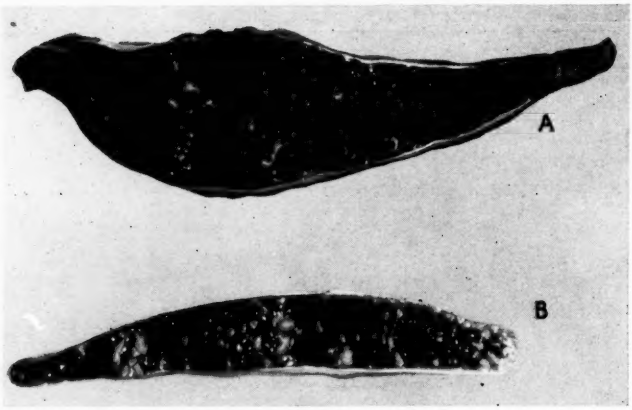


Fig. 1
A.—Cross section of slaughter spleen of ox induced by vigorous manipulation of an abnormally long pithing cane. B.—Cross section of normal ox spleen.

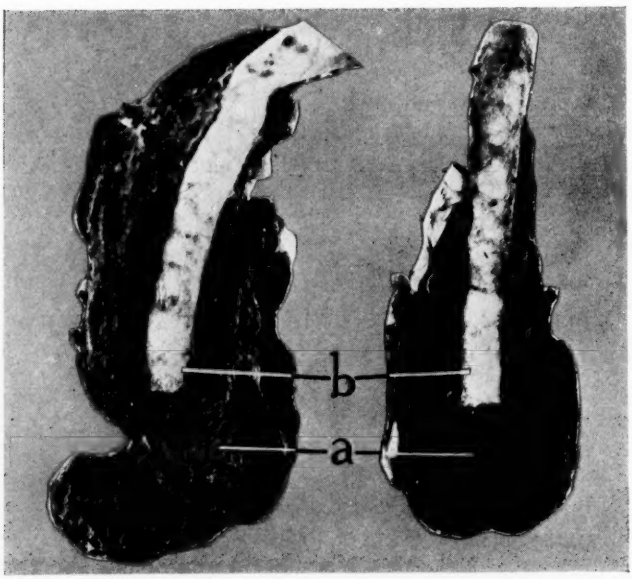


Fig. 2
Section of proximal ends of two carotid arteries which have been severed during ritual slaughter of an ox. Retraction of the artery has caused the loose connective tissue (a) to 'bunch' around the cut end of the vessel (b), thus forming a seal.

(Article by H. Thornton, on page 164)

in animals and that this information can be taken advantage of by the research worker in the laboratory. It is true that carefully kept records, not always an easy matter at the time of peak kills, give a useful picture as to the incidence of diseases such as tuberculosis, fascioliasis or hydatidosis in our domestic stock and enables us to assess the economic losses for which they are responsible.

Admittedly desirable as a liaison between the abattoir worker and research worker may be, there is little evidence in Britain that any such liaison exists at the present time. The inspector in the rural district who is responsible for the examination of carcases in a number of widely scattered slaughterhouses is in a particularly unenviable position, for his relative isolation gives him little or no opportunity for discussion and elucidation of the more puzzling conditions he encounters. Yet his decisions are often of the greatest public health importance, inasmuch as being in a rural district he will be presented with numerous cases of animals which have been slaughtered in emergency for various causes.

Let it be said at once that if meat inspection is conducted by a person who is thoroughly interested in the subject he will encounter problems almost daily which he will find of absorbing interest, whereas if meat inspection is carried out as a dull daily task there can be no public health duty which can be so soul-destroying. The purpose of this contribution is to draw attention to some phenomena and conditions which the writer has encountered during the years in the slaughterhouses in Newcastle upon Tyne and to appeal for scientific investigation by research workers into their ætiology. Suggestions are made as to the possible causes, predisposing and otherwise, of the conditions, but it is realised only too well that such suggestions cannot be other than empirical and tentative pending thorough scientific investigation.

1. The So-called Slaughter Spleen in Cattle and Pigs

The spleen is an organ better able than other organs to hold vast quantities of blood because of its sinusoids and it reacts extraordinarily rapidly to nervous excitation. It is also important to realise that in the living animal the spleen is a very large organ, very much larger than when it is seen on routine post-mortem examination, for when an animal is slaughtered the throes of death and loss of blood due to sticking usually causes the spleen to contract to minimal proportions.

Some few years ago in one particular slaughterhouse it was found that during post-mortem inspection of the cattle slaughtered a number of these exhibited enlarged and congested spleens. The cut surface appeared to bulge, revealing a dark red pulp from which blood flowed out readily (Plate 1) and sometimes only an odd spleen was encountered during a day's kill, but sometimes a whole batch of cattle slaughtered on a particular day showed this splenic enlargement. The fact that the carcases of the animals were normal in every way and that the possibility of anthrax was ruled out by microscopical examination showed that this abnormality was probably connected in some way with the act of slaughter. In British publications the literature dealing with splenic enlargement due to causes other than those of an infectious nature was meagre in the extreme and confined to the pig. Allen (1937) stated that in pigs the spleen is often enlarged without any other lesion in the carcase, while Anthony (1940) mentions that splenic congestion is often seen in

abattoir inspection and is especially well marked in the large spleen of adult pigs and sows. Runnells (1946), however, states that in abattoirs it is observed in normal animals after slaughter and that though the cause is not known it is thought to be due to splanchnic influences.

One could find no reference in British literature to congestion of the spleen in cattle due to mechanical causes but after seeking enlightenment in German publications one was soon convinced that the condition noticed in one's own city was identical with that occurring in Germany in both cattle and pigs and known as " bloody spleen " or " slaughter spleen ". An extensive discussion on this subject appeared in Volume 22 of the Zeitschrift fur Fleisch und Milchhygiene for 1912 and these observations are of such interest that they are summarised below. Klepp, Veterinary Director of the Potsdam Abattoir, described encountering a bovine spleen twice as broad and twice as long as normal, and though the organ was greatly thickened and markedly congested the splenic tissue itself was normal in texture and firm. The liver was congested and of a brownish-black colour, though it was firm in consistency and not friable, while the kidneys, particularly the cortex, were also congested with blood. (These changes in the liver and kidneys are significant and will be referred to again in this article.) Klepp noted that the carcase was normal in every respect but he was understandably mystified by the condition and contented himself by describing it as an " undefined disease of the blood ".

During the discussion, Junack, of the Cottbus Abattoir, remarked that he had noticed the condition of " slaughter spleen " in both cattle and pigs, occasionally in whole batches of pigs, and suggested that it might be caused by the older type of Behr shooting instrument, a pistol of the free bullet type which was used extensively in Germany for the slaughter of all types of animals at that time. He suggested that the shock induced by this form of stunning was due to the use of too strong a cartridge in the pistol and that this caused congestion of the internal viscera, a large part of the systemic blood becoming localised in the spleen and in the veins of the organs situated in the lower parts of the abdominal cavity. Junack, like Klepp, noticed that the liver was in some cases congested and in some cases he also found the intestines so congested that they had the appearance of an animal which had died naturally. He makes the significant remark that the veins of the portal system, mostly possessing no valves, readily permit the return of blood from the veins into the organs, such as the intestines and spleen, which they drain.

Stroh, Senior Veterinarian of Augsburg, also recorded seeing the condition regularly in cattle, and stated that to the naked eye the spleen bore a striking resemblance to that of a spleen from an animal dead of anthrax; he had noticed that sometimes the enlargement was confined to the thicker dorsal portion of the spleen, he also had noticed the occasional congestion of the liver and stated that when " slaughter spleens " occurred in calves there was always a marked congestion of the small intestines, though the large intestines appeared normal. In the Augsburg slaughterhouse in the year 1906, Stroh encountered slaughter spleens in five oxen, one bull, three cows and ten calves; in 1909, in five oxen and three cows, and in 1910, in ten oxen, two bulls and two calves. Another municipal veterinarian stated that he had formerly had all pigs slaughtered by a club, but had then directed

that these animals be despatched by a shooting instrument; almost immediately he encountered numerous cases of what he described as " traumatic spleen tumour ", together with congestion of the liver and intestines, and as a result of these untoward changes he had received a deputation from the butchers asking permission to revert to the stunning of pigs by means of the club.

From the above observations there seemed to be little doubt that the term slaughter spleen was a correct one and was related in some way to the method of slaughter and that the spleen, instead of contracting as it normally does when the animal is bled to death, had undergone dilatation and remained suffused with blood. Professor Schönberg, of Hanover, in reply to queries from the writer, stated that slaughter spleen or " traumatic spleen tumour " as it is sometimes termed in Germany, had often been observed in that country in cattle and pigs stunned by captive bolt instruments and suggested that the condition occurred during inefficient stunning when there was damage to the sympathetic nervous system. (He does not state whether the pithing cane is in common use in Germany during the slaughter of bovine animals, but in Zurich Abattoir a pithing cane is used, though this is only 24 inches in length.) Professor Mangold, in a reply to Professor Lerche, to whom I wrote in Berlin, pointed out that the vaso-constrictor centre of the sympathetic nervous system lies in the medulla oblongata, and destruction of the medulla oblongata will inhibit this vaso-constrictor action and permit peripheral dilatation of the blood vessels and general lowering of the blood pressure. This fall in blood pressure results from dilatation of the splanchnic blood vessels which supply the spleen, liver and intestines and it is significant that the German observers referred to earlier in this article remarked on the congestion, not only of the spleen, but of the liver, kidneys and intestines. There can, therefore, be little doubt that the condition is not a local one which is confined to the spleen alone but is a general one in which the spleen shows the greatest and most striking change.

The possibility that damage to the sympathetic nervous system, either in the brain itself or in the spinal cord, might cause dilatation of the splanchnic vessels and the production of slaughter spleens, focused the attention of the writer on the effects of the pithing cane. The practice of pithing entails the insertion of a long bamboo cane, sometimes a long wire, into the hole made in the frontal bone of cattle by the captive bolt, the cane being then moved to and fro to destroy the medulla; the purpose of this practice, which is common in Britain but is less common in other countries, is to destroy reflex action of the part of the animal when dressing of the carcase is commenced and it is therefore a technique directed purely towards the saving of the slaughterman's time. Enquiries at the slaughterhouse in the city where the slaughter spleens in cattle had first been noticed elicited a most significant piece of information; the slaughtermen had recently been supplied with a new set of pithing canes, these being five feet in length instead of the four-foot canes generally used.

In consideration of this problem it should be realised that the great splanchnic nerve has its origin from the nerve roots which arise from the spinal cord between the fifth and eleventh vertebræ, and as the distance from the frontal bone along the spinal canal to the fifth dorsal vertebra is only 32 inches in an average-sized

animal, a five-foot cane fully inserted will reach the fourth lumbar vertebra and will destroy all the nerve roots arising from the thoracic portion of the spinal cord (Fig: 1). The splanchnic nerve is the great vaso-constrictor of the abdominal cavity and it can be demonstrated experimentally that complete section of it will cause dilatation of the abdominal blood vessels, which will become filled with blood. It would be expected that destruction of the roots of the great splanchnic nerve between the fifth and eleventh dorsal vertebræ by pithing would operate similarly and that the further the cane is introduced into the spinal cord and the more vigorously it is manipulated the more likely would it be for reflex action to be completely inhibited. This complete inhibition of reflex action was the reason that the long cane commended itself to the slaughtermen, who stated " these canes are the best thing that was ever introduced into the slaughterhouse, they kill the animal at once ". It therefore appeared that the abnormally long cane was causing the production of slaughter spleen, and the pithing canes were shortened, on our instructions, to half their length and the occurrence of slaughter spleens ceased forthwith.

VASO-CONSTRICTOR NERVE SUPPLY TO THE ABDOMINAL ORGANS.

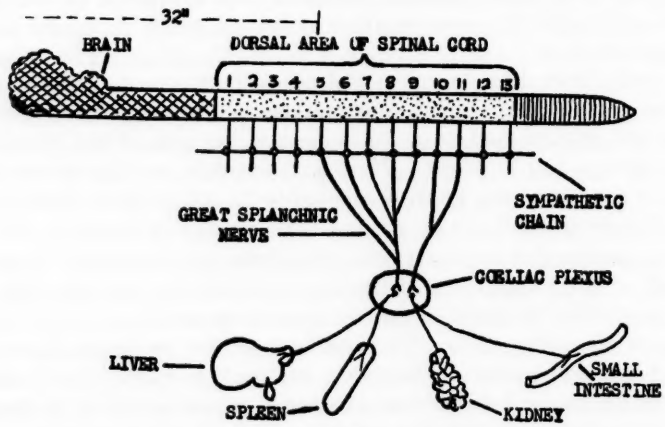


Fig. 1

Diagram showing the origin of splanchnic nerves from 5th—11th dorsal segments of the bovine spinal cord, and the distribution of vaso-constrictor fibres to the spleen and other abdominal organs. Note that a pithing cane introduced into the spinal canal to a depth of 32 inches will reach the fifth dorsal vertebra

On occasions, odd slaughter pleens have occurred even with the use of the shortened pithing canes, and an ætiological factor in such cases may be that if cattle are shot and bled without the introduction of the pithing cane the reflex muscular contractions will aid in squeezing out blood from the spleen, whereas where reflex movement is absent, as in adequately pithed animals, no such squeezing action will occur. In other words, animals shot and not pithed are less likely to show slaughter spleens than are animals which are pithed and it is perhaps significant that slaughter

spleens do not appear to have ever been recorded in animals slaughtered by Jewish ritual means.

If the absence of reflex muscular action in cattle which are pithed is a factor in producing the condition, this may also provide an explanation of why slaughter spleen is sometimes observed in pigs slaughtered by electrical means, for in this method of slaughter the inco-ordinated reflex actions which are associated with the use of the captive bolt pistol are noticeably absent. In addition, the observation of Stroh that sometimes only a portion of the spleen in cattle is congested (an observation confirmed by ourselves in Newcastle upon Tyne) would lead one to suspect that still other factors may play a causative role in the occurrence of slaughter spleen. Factors which might therefore be investigated are:

(a) The effect of an inadequate period of rest prior to the animal being slaughtered,

(b) The time interval occurring in cattle between shooting and pithing.

(c) The diameter of the cane, and whether the pithing is unduly prolonged. A thin wire may do less damage to the spinal cord than a malacca cane.

(d) The interval between pithing and bleeding and whether the animal is bled " down hill ", i.e. with the head lower than the hindquarters, and whether the animal is " possed " after the cutting of the neck vessels. If there is an undue interval between pithing and bleeding, the spleen may have " set " in the dilated condition by the time bleeding is commenced.

(e) The side on which the animal falls when shot, for the abdominal organs may exert pressure on the posterior vena cava and thus cause certain organs, including the spleen, to become congested.

From the few experiments we have been able to make we are led to the conclusion that the prime causative factor of slaughter spleens is the vigorous use of a long pithing cane, and using a cane which penetrated 43 inches we have been able to produce the condition in slaughtered cattle provided the cane was so manipulated as to ensure a thorough destruction of the spinal cord and the nerve roots arising from it. The factors (a), (b), (d) and (e) mentioned above have, in our experiments, proved to be of little or no ætiological significance.

As slaughter spleen sometimes occurs in sheep stunned by electrical means and is commoner in lambs than in older sheep, investigations might also be made into the occurrence of this condition in these animals, attention being paid as to (a) the age of the slaughtered animals, (b) whether the condition might not be associated with too high a voltage passing through the animal's brain, and (c) whether the breaking of the neck after electrical stunning (a practice directed towards the obviation of reflex muscular action and therefore with a similar purpose to that of the pithing of cattle) may be an ætiological factor in this condition. The occurrence of slaughter spleens in sheep under somewhat unusual but interesting conditions may be recounted here. The writer visited Peru in 1952 and was furnished with details as to the preparation of " Chalone ", which consists of mutton dried in the sun. The slaughter of the sheep takes place in the Peruvian Highlands at a height of some 15,000 feet, the carcases being dried in the hot sun during the day and exposed to the cold frosty air during the night, yielding a product which will keep well even

when sent down to the hot forest regions. The natives, mostly inebriated on a drink known as " Chicha " which is made from the fermented juice of the Yucca plant, will kill from 3,000 to 5,000 sheep in a night, and one was informed that the dressing of the animals is commenced the moment the throat is cut and before the animal has died. In spite of the fact that the muscular reactions of sheep slaughtered in this manner would be expected to be severe and uninhibited, it has been observed that many of the slaughtered animals exhibit enlarged and congested spleens.

Critical research into the possible causative factors of slaughter spleens in the various animals would be of the greatest value, for in meat inspection there is always the danger that what appears to the inspector to be a slaughter spleen may in actual fact be the spleen of an animal affected with anthrax or some other acute septicæmic condition.

2. Sealing of the Carotid Artery in Cattle Slaughtered by the Ritual Method

As far back as 1904 a Committee was appointed by the Admiralty to consider the humane slaughtering of animals and in its report the Committee found that " all animals without exception must be stunned or otherwise rendered unconscious before blood is drawn ", while with regard to the Jewish method the Committee felt " that consideration of humanity must be regarded as paramount and that no unnecessary suffering could be condoned on the ground that it was incidental to the observance of any religous custom ". The Committee stated they had been forced to the following conclusions " (a) that the Jewish system fails in the primary requirements of rapidity, freedom from unnecessary pain and instantaneous loss of sensibility, (b) that the preliminary operations of casting and of forcing the animal's head into position for the cut are difficult, painful and objectionable from a humanitarian standpoint, and (c) that the subsequent operation of cutting the throat is at best an uncertain method of producing immediate loss of sensibility and frequently causes great and unduly prolonged suffering to the animal ".

The conclusions of the Committee were arrived at after receiving the report of two eminent physiologists, Sir Michael Foster and Professor Starling, but against this must be weighed the testimony of other experts such as Professor Lovatt Evans and Sir Leonard Hill who investigated the problem of Jewish slaughter and were of the opinion that the method was swift, painless and humane.

In the consideration of this problem no more than a passing reference need be made to the objection in the Admiralty report as to the method by which the animal is prepared for Jewish slaughter. The passing of legislation in Britain, namely the Slaughter of Animals (Prevention of Cruelty) Regulations, 1954, which requires the use of an approved type of casting pen for the slaughter of adult cattle for Jewish consumption, has removed one of the undoubted objections to the Jewish method of slaughter. On the other hand, the statement made by the Committee that the subsequent operation of cutting the throat " is at best an uncertain method of producing an immediate loss of sensibility " is a matter on which controversy has raged for many years. The purpose of this contribution is to remind the research worker that there are now techniques available by which the existence of con-

sciousness can be accurately demonstrated by electro-encephalographic means and also to draw the attention of the research worker to a phenomenon which occurs commonly in ritually slaughtered cattle, namely the sealing of the cut end of the carotid arteries. It is suggested that this sealing may be of such significance in prolonging the period of consciousnes that it warrants immediate investigation.

The problem of Jewish slaughter is reviewed under the following headings:—

(a) The reactions of the live animal,

(b) The neck cut,

(c) The blood supply to the ruminant brain,

(d) Sealing of the carotid artery and its possible effects in prolonging consciousness,

(e) The assessment of the duration of consciousness by electro-encephalographic means.

(a) *The Reactions of the Live Animal*

The view of the hardened slaughtermen and that of the sentimental layman as to whether or not an animal in a slaughterhouse is aware of its impending death are so diametrically opposed that no scientific or commonsense arguments on the question are likely to receive unbiased consideration by either side. There is little doubt that the correct approach to this problem lies midway between the two opposing camps. There can be no justification whatsosever for rough treatment of an animal, as, for example, in inducing it to enter a Jewish slaughter pen. On the other hand, experience in a slaughterhouse leads the trained veterinary surgeon to the view that the sight or smell of blood does not produce a feeling of terror in the animal; strange noises and strange surroundings, however, do disturb animals and for that reason it is essential that when cattle are slaughtered ritually in a casting pen, the pen should be rotated immediately the animal enters and the neck cut made as rapidly as possible; this latter requirement applies also to sheep in the ritual slaughter of which a stunning pen is not required.

(b) *The Neck Cut*

In the ritual slaughter of cattle the pen is rotated so that the neck of the animal is uppermost and the neck is then cut by a transverse incision which severs the skin, muscles, œsophagus, trachea, carotid arteries and jugular veins. (The vertebral artery, which runs upwards in relation to the cervical vertebræ and then along the floor of the spinal canal, of course, remains intact.) Shortly after the neck cut is made, the animal commences to exhibit convulsive muscular movements but no physiologist would suggest that such movements are connected in any way with the consciousness of the animal; likewise the persistence of the corneal reflex, frequently advanced by opponents of Jewish slaughter as evidence of consciousness, is not associated with consciousness and its significance can also be discounted. These observations stress the point that the opinions expressed in many of the earlier writings on Jewish slaughter were largely valueless because they were only opinions and had not been substantiated by carefully conducted scientific experiments.

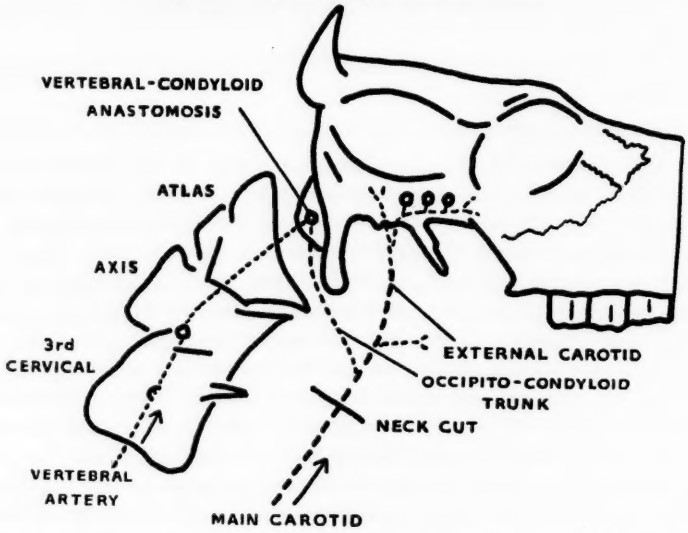
(c) *The Blood Supply to the Ruminant Brain*

The major blood supply to the brain is by the branches of the main carotid

artery, namely the external carotid and internal maxillary artery (Fig. 2). Branches from the latter vessel penetrate a number of foramina in the skull and enter the arterial network known as the rete mirabile, and the rete gives off vessels which form the Circle of Willis, the arterial structure which supplies blood vessels to the actual substance of the brian. With one exception, the vessels running into the

Fig. 2

Arterial blood supply to the brain of the ox. This supply is almost entirely by the main carotid trunk and external carotid artery, but when the main carotid is cut or occluded a supply may still reach the external carotid by way of the vertebral artery and occipito-condyloid trunk.



rete mirabile are branches of the main carotid artery, one on each side of the neck, and the carotid arteries are therefore the most important vessels concerned in the blood supply of the ruminant brain and are severed completely as a result of the knife cut in Jewish slaughter. The vertebral artery is the exception, for it passes up the neck and then along the floor of the spinal canal formed by the cervical vertebræ and is therefore not severed during Jewish slaughter. By anastomosis of the vertebral with the condyloid artery, the latter a branch of the occipital artery which again is an important branch of the carotid artery, an artery is formed which penetrates a foramen at the base of the skull and enters the rete mirabile, but this artery, the basilar, is so small that the amount of blood it supplies to the rete mirabile is of little or no importance. The vertebral artery, however, because of the large anastomosis it makes with the condyloid, is a potential source of supply to the brain after the carotids are occluded, for blood from the vertebral artery then flows along the occipito-condyloid trunk in a reverse direction and into the external carotid artery. When the carotids are severed an immediate fall in carotid pressure occurs, and a fall, but more gradual, occurs in the pressure in the vertebral artery so that for a time a supply of blood continues to the brain by way of the vertebral-

condyloid anastomosis and external carotid. It is doubtful whether this supply is sufficient to maintain consciousness after complete severance of the carotid arteries. A point of extreme importance, however, is as to what may occur to the blood supply to the brain if the carotid arteries are not severed but the flow in them is interrupted, as might be done experimentally, by ligation.

Linzell and Waites (1957) have shown by experiments on sheep and goats that ligation of both carotid arteries in eight animals produced unconsciousness in one animal only, and they conclude therefore that when both carotid arteries were occluded, blood from the vertebral arteries was entering the external carotid artery above the point of occlusion through the large vertebral-condyloid anastomosis and thus reached the brain via the rete mirabile by the normal route. They conclude also that under all conditions the external carotid arteries would appear to be the final common path for blood to the Circle of Willis. It is also of importance to consider what may occur in the vertebral artery when there is a sealing of the cut ends of the carotid arteries as sometimes happens shortly after the ritual neck cut is made.

(d) *Sealing of the Carotid Artery and its Possible Effects in Prolonging Consciousness*

In order to consider this phenomenon it is necessary to appreciate the anatomical structure of the larger arterial blood vessels. An artery is usually described as being composed of three coats, an inner or elastic coat, a middle or muscular, and an external or areolar, but it is more correct to describe the wall of an artery as being mainly composed of muscular and elastic tissue, lined internally by a pavement epithelium and strengthened externally by a layer of connective tissue. In this external layer (the tunica adventitia) the outer limit is not sharply defined for it blends with the surrounding connective tissue. In the carotid artery, the vessel which is cut during Jewish slaughter, the following structures may be distinguished (Fig. 3):

(a) An inner layer of endothelium, continuous with the endocardium,

(b) A coat of yellow elastic very closely packed fibrous tissue,

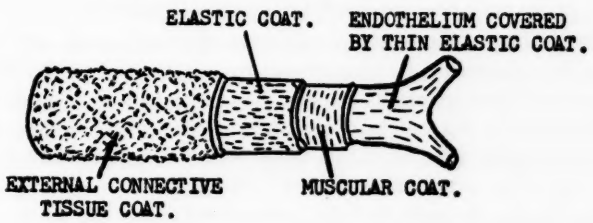
(c) A coat of white muscle fibrils arranged as a spiral around the lumen, so that on contraction the lumen of the tube is reduced,

(d) Another coat of yellow elastic tissue, similar to (b), and

(e) A coat of loose areolar tissue.

Fig. 3

Diagram showing coats of the carotid artery.



In the large arteries near the heart, such as the carotid, the elastic coat is thick and strong and the artery remains patent event when empty, but when the carotid is severed completely by a transverse cut with a sharp knife there is an immediate retraction of the proximal and distal ends leaving a wide gap between the two. It will be appreciated that the more dexterous the cut and the greater the extension of the neck at the time of the cut, the greater will be the retraction of the vessel itself. From the proximal end of the carotid artery blood continues to gush and if this hæmorrhage is uninterrupted and the heart continues to function the animal will rapidly become exsanguinated, anoxia of the cerebral nerve cells will occur and unconsciousness will naturally supervene.

The flow from the proximal end of the carotid artery is not invariably an uninterrupted one, for on retraction of the severed ends of the carotid artery there is a curling of the endothelie lining and the outer loose areolar tissue is torn and " bunches " around the proximal end of the cut vessel; this may act as a seal and prevent further escape of blood from the artery and in some cases were have observed this to occur very rapidly after the neck cut is made (Plate 2). As a result of this sealing the blood pressure in the anterior aorta will be maintained by (*a*) the continuance of function of the heart, and (*b*) by the immediate constriction of the blood vessels that follows a severe hæmorrhage, and the blood pressure in the vertebral artery will likewise be maintained at a substantial level; thus if sealing occurs rapidly the blood supply to the brain by way of the vertebral artery and the occipito-condyloid trunk may be sufficient to ensure that consciousnes is maintained.

The rapid sealing of the cut ends of the carotid artery does provide an explanation as to why cattle, the throats of which have been cut dexterously and efficiently by the Jewish method, have on occasions been observed to break their hobbles and regain their feet and make for the open door of the slaughterhouse, walking some distance before they have eventually succumbed. In the past when such occurrences have been witnessed they have been attributed, perhaps for want of a better explanation, to a miss-cut, i.e. that all the neck vessels have not been severed completely, but experience shows that in Jewish ritual slaughter the efficiency of the neck cut is only rarely in question and that what has actually happened in such cases is that there has been a retraction and rapid self-sealing of the carotid arteries thus ensuring a substantial supply of blood to the brain by way of the verebral-condyloid anastomosis.

By the very nature of the neck cut made in Jewish slaughter of cattle it is impossible to ensure that self-sealing of the cut ends of the carotid arteries will not occur, and slaughtermen who are employed to assist the " Schochet " or cutter in his cutting of the neck have proffered the following significant observations :

(*a*) That sealing of the blood vessels takes place frequently and in order that bleeding can be expedited the slaughterman frequently cuts off the sealed end of the carotid so that bleeding may be resumed. In some cases the Jewish " Schochet " raises no objection to this but in other cases a " Schochet " who is actuated by rigorous ritual scruples will forbid this to be done. The slaughtermen say " it all depends on who is the cutter whether this will be allowed ".

(*b*) The sharper the knife and the more expeditiously the cut is made by the

" Schochet " the more likely is sealing of the carotids to take place.

(*c*) Sealing is more likely to take place in horned cattle than in polled animals. A logical explanation for this is that in horned cattle it is possible for the slaughterman to grasp the horns and stretch the neck to a greater extent than is possible in polled animals. The stretching of the neck is an essential operation in Jewish slaughter, and due to the greater extension in horned cattle the retraction of the severed carotid will be more forcible and sealing will be more likely to take place.

(*d*) Although both carotid arteries may seal shortly after the neck cut, the right carotid seals more frequently and usually seals first. If one studies the arrangement of the Jewish slaughter pen one will notice that when the pen is rotated the animal is lying on its right dorsal aspect; the slaughterman, standing to the left of the animal, must pull in a somewhat sideways direction and the result is that the right side of the neck is stretched more than the left side.

These personal observations have been made by slaughtermen who have had many years of assisting at the neck cut in the Jewish slaughter of cattle.

e. *The Assessment of the Duration of Consciousness by Electro-encephalographic Means*

Though the physiological basis of consciousness is still in dispute, recent research has now established the fact that the state of consciousness in the higher animals can be determined by measuring the electrical activity of the brain, for the pattern of activity changes as consciousness decreases in sleep or anæsthesia. By the placing of unipolar electrodes under the skull but external to the dura mater through trephine holes over defined parts of the cerebral cortex and connecting electrodes in pairs to a two-channel electro-encephalogram it is possible to obtain a record of the functional activity of the cerebral cortex during the various stages of slaughter.

In laboratory animals placed under light nembutal anæsthesia observations can be made on the electrical activity of the central nervous system and on the heart rate, and electro-encephalographs from the brain, which indicate whether an animal is conscious or not, are actually one of the few methods available for detecting consciousness in a paralysed animal. Electro-encephalographic records made from a normal conscious rabbit, the records being obtained from the pre-central granular area of the cortex, show a regular rhythm of six to eight cycles per second (Croft, 1952). During light anæsthesia this rhythm appears in intermittent bursts, while when the animal is completely unconscious due to anæsthesia the rhythm disappears completely.

The changes that take place in the cortical activity of animals " bleeding to death " are very similar to those which take place during the onset of sleep. For a period after the cut is made no change in electrical activity is discernible; this is followed by a decrease in high-frequency low-amplitude waves and an increase in waves of low frequency and higher amplitude. Towards the end of this period there is a marked and progressive reduction in electrical activity and this may represent the onset of loss of consciousness. Finally, there is a prolonged period during which no electrical activity at all is to be seen.

Though some experiments carried out on laboratory animals have indicated

that consciousness is lost in two or three seconds after the complete severance of the carotid arteries, on the other hand it has been established that stopping the circulations in cats, dogs (Roberts, 1954) and man does not cause loss of consciousness for at least twelve seconds. It is considered that the cerebral cells may obtain oxygen for normal metabolism during this period without any fresh supply of blood, and Croft records that unpublished work by other scientists shows that similar results are found in the cow.

Because of the presence of the vertebral-condyloid anastomosis and its functional role in supplying blood to the brain via the external carotid artery it is suggested that cerebral anæmia may not occur rapidly after the neck vessels are severed, while when sealing of the cut carotids occurs the period of consciousness may be appreciably longer, and certainly longer than has hitherto been supposed. The question of the duration of consciousness in ritually slaughtered animals may possibly be solved by encephalographic records taken prior to the neck cut being made, while the neck cut is being made, and during subsequent bleeding.

To be concluded in the June issue of this Journal

(The references will appear at the end of Part II of this article)

THE PRODUCTION OF BOVINE HÆMORRHAGIC SEPTICÆMIA VACCINE BY CONTINUOUS CULTURE

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THE yield of *Pasteurella multocida* in unaerated liquid medium is approximately 10^9 organisms per ml. (0.1 gm. dry weight per litre). Since about 2 mg. of killed, antigenically satisfactory organisms are needed to immunise a bovine animal (Bain, 1957) an inconveniently large dose of a formalised culture of this density would be needed to elicit a satisfactory immunity. Bain (1955) increased the yield and concentration of organisms by aerating the medium and thus prepared vaccines which, when emulsified in lanolin and oil, were effective in a dose of 3 ml. Shirlaw (1957) achieved a similar result by concentrating unaerated cultures of *Pasteurella* in a Sharple's centrifuge. By using more thorough aeration we were able to increase the yield to approximately 20×10^9 viable organisms per ml. (Brown's tube 30 opacity, dry weight 2-4 gm/litre), so that an effective immunising dose could now be included in 1 ml., and approximately 1,000 doses obtained from a litre of culture.

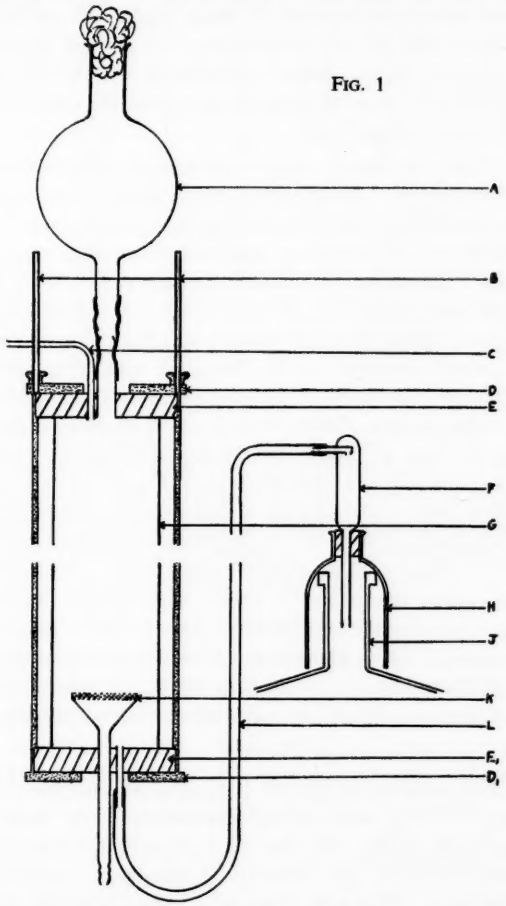
Attempts to increase yields further were only partly successful. When the carbohydrate content of the medium was increased the pH of the culture fell rapidly and growth ceased below pH 5.6. When the carbohydrate content was reduced, the pH rose steadily. When the pH exceeded 7.6 a mucoid substance separated from the culture and formed a thick scum which entangled the organisms. This scum did not redissolve on acidification.

Because of the short generation time of *Pasteurella multocida* during logarithmic growth, it seemed likely that harvests dense enough for practical use could be obtained by continuous culture, and that the concentration of carbohydrate and the flow of medium could be balanced to give a steady hydrogen ion concentration. These hopes were fully realised in practice, and all the hæmorrhagic septicæmia vaccine prepared at these laboratories is now produced by continuous culture*.

Methods

Strain. The hæmorrhagic septicæmia vaccine (for use against bovine plague

FIG. 1



in East Africa) is prepared from three strains of *Pasteurella multocida* Type 1 (Roberts). One of the strains is of Asian and two of African origin. Freeze-dried

* Towards the end of this work we learned that Dr. Bain, of Sydney University (Dr. Bain, pers. com.), was working on very similar lines and using an apparatus for continuous culture of *Pasteurella multocida* which was similar in dimensions and performance to that described here.

cultures are reconstituted and injected into rabbits. At death, their blood is streaked on to agar plates, fluorescent colonies selected, grown, and again injected into rabbits. This procedure is repeated until the cultures have a mouse virulence of 5 to 10×10^8 MLD/ml. These selected passaged strains are used for seed.

Medium. Nutrient broth to which has been added

Autodigest of pancreas to 12.5 per cent.

Glucose to 0.2 per cent.

Sodium lactate 0.2 per cent. (Jordan, 1952).

Sodium dihydrogen phosphate 0.3 per cent.

Adjust pH to 7.5, heat to 60° C., filter, add 2 ml. of 10 per cent. laked bovine blood per litre of medium, and sterilise by filtration through Ford's S.B. pads. Before inoculating add 10 ml. of 20 per cent. NaH_2PO_4 per litre of medium and antifoam (Dow-Corning antifoam A in technical light oil).

Apparatus. This is extremely simple and requires only the crudest workshop facilities for its construction. It differs from the similar equipment described by Herbert, Elsworth and Telling (1956) in not having a stirrer, since "instantaneous" mixing was not primarily of importance and sparger aeration was adequate for the volume of medium. The culture vessel G is a pyrex tube 760×114 mm. closed at each end by rubber discs, E and E', 30 mm. thick. These are clamped on to the ends of G by means of brass plates, D and D', which are drawn together by means of three equally spaced threaded rods B, furnished with wing nuts. The medium enters by tube C and may be fed by gravity or, preferably, pumped over, and leaves by tube L to be collected in a 15-litre flask J. F is an anti-siphoning head which makes possible an accurate adjustment of the height of the medium in G. H is an anti-contamination hood. K is a sparger formed of a No. 3 sintered filter. A is a one-litre boiling flask with a tube 25 mm. in diameter fused into the bottom connecting it to the culture vessel by way of a short piece of wide bore rubber tubing.

Operation. G is filled with medium (through C) to a level determined by the height of F. The volume is usually five litres. The medium is inoculated through A with a vigorously growing six-hour culture which has been passaged and selected as described previously. Extra phosphate and antifoam are added at the time of inoculation. A vigorous flow of air (1,000 cc/minute) is started through K. After 18 hours, a flow of medium of 1,000 to 1,200 ml. per hour is started.

Results

So long as active exponential growth is maintained and the pH is kept reasonably constant, there is little tendency to dissociation. The results of a series of harvests are given in the table. The flow of 1,200 ml/hour was decided on for the sake of convenience to avoid the necessity for changing over to a fresh flask of medium during the night. However, flows of 2,500 to 3,000 ml/hour gave a better control of pH as well as the highest opacities registered (50 Brown's tube).

Discussion

The five-litre culture vessel described above produces approximately 30,000 doses (at 2-5 mg. dry weight or organisms per dose) per 24 hours. The output

TABLE 1

The opacity, the number of viable organisms and the dry weight of organisms in samples taken at intervals from continuous cultures of Pastuerella multocida Type I (Roberts).

Strain	Batch	Opacity (Brown's tube)	Viable count (per ml x 10^{-9})	Dry weight in gms/litre
CN 3780	89/2	27	28 ± 3	2.95
CN 3780	89/3	27	29 ± 3	3.6
CN 3781	90/2	30	30 ± 2	3.8
CN 3781	90/3	24	36 ± 3.4	-
CN 3782	91/1	25	36 ± 2	3.4
CN 3782	91/2	24	32	3.0
CN 3782	91/3	27	24 ± 1.8	2.85
CN 3782	91/4	32	31 ± 2.1	3.18
CN 3782	92/1	21	27.5	3.0
CN 3782	92/2	18	28 ± 2.4	2.1
CN 3782	92/3	30	45 ± 2	3.35
CN 3782	92/4	30	-	2.7
CN 3780	94/1	25	19 ± 1.4	2.15
CN 3780	94/2	27	46 ± 2	3.45
CN 3780	94/3	28	31 ± 5	2.5
CN 3780	94/4	28	54 ± 3	3.0
CN 3781	95/1	24	32 ± 2.3	2.7
CN 3781	95/2	24	28 ± 2.2	2.9
CN 3781	95/3	28	32 ± 2.3	4.1
CN 3781	95/4	24	31 ± 2.3	3.2
CN 3782	99/4	50	56 ± 3.3	4.74

could, however, be raised to more than 100,000 doses per day by simply running the apparatus to capacity. This production could probably be doubled by doubling the volume of the culture vessel. Any increase beyond this would almost certainly require more efficient aeration and, consequently, a more elaborate culture vessel; such as, for example, that described by Herbert *et al.* (1956).

Summary

A small continuous culture vessel has been used for the routine production of hæmorrhagic septicæmia vaccine (*Pasteurella multocida*, Roberts Type 1). Harvests of 30,000 doses per day at a concentration of 2-5 mg. dry weight of organisms per dose have been obtained regularly. When run at full capacity, the apparatus could produce more than 100,000 doses per day. Dissociation was negligible.

Acknowledgement

We are grateful for the help given during the earlier part of this work by Mr. D. Weatherby, of these laboratories.

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STUDIES ON BRACKEN POISONING IN CATTLE

PART IV

By W. C. EVANS, M.Sc., Ph.D., F.R.I.C., I. ANTICE EVANS, Ph.D., A. J. THOMAS, M.A., Ph.D., J. E. WATKINS, M.A., Ph.D. and A. G. CHAMBERLAIN, B.Sc.

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THE inclusion of fresh, green or air-dried bracken in the diet of simple-stomached animals, e.g. the rat or horse, gives rise to an avitaminosis B_1 (Weswig, Freed and Hagg, 1946; Evans, Evans and Roberts, 1949, 1951). This condition arises as a result of the presence of thiaminase in bracken (Evans, Jones and Evans, 1950, 1952).

Cattle bracken-poisoning has been described in detail previously (Evans, Evans and Hughes, 1954; Naftalin and Cushnie, 1954); the syndrome in the ruminant is entirely different from that which occurs in homogastric animals. The predominant feature is a depression of bone-marrow activity, giving rise to a severe leucopenia and thrombocytopenia; there is no avitaminosis B_1. The mechanism of production of this lesion is not known—whether thiaminase is somehow involved, or whether bracken contains yet another harmful agent has remained a mystery.

It might be surmised that if bracken contains a second factor, toxic to bone-marrow, then simple-stomached animals should be potentially susceptible to its action, if conditions were not masked by the earlier onset of avitaminosis B_1.

The production of the characteristic hæmatological lesion found in the cattle disease, was more difficult to accomplish in sheep (Moon and Rafat, 1951; Moon and McKeand, 1953). In rats, Naftalin and Cushnie (1951) mention that lesions resulting from destruction of the bone-marrow on feeding bracken, were mediated by a vitamin B_1 deficiency. Subsequently, Cordy (1952) published hæmatological data showing that rats on a ration containing raw bracken-fern had a thrombocytopenia, some leucopenia and a marked bone-marrow hypoplasia; thiamine therapy also promptly corrected these changes.

These workers therefore attributed the bone-marrow damage in bracken-fed rats to the avitaminosis B_1 producing property of the plant, rather than to another toxic principle.

Another puzzling feature is the absence of a demonstrable thiamine deficiency in the tissues of cattle poisoned by bracken. Conditions in the rumen, with a pH near neutrality, are favourable for thiaminase action, and this is reflected by the absence of any extracellular thiamine in rumen liquor of cattle fed a bracken diet (Phillipson and Reid, 1954). Results to be described in this communication makes it clear that there is still an ample rumen flora in such animals—indeed, not differing appreciably from that of animals on a normal diet. In the abomasum, this microflora would be autolyzed and digested, with the liberation of previously intracellular thiamine and its physiologically active derivatives. This region of the alimentary tract, being acidic, is unfavourable for thiaminase action. It is also possible that a considerable amount of destruction of thiaminase occurs in the rumen by microbial

proteolytic enzymes, so that when the ingesta arrives at the true stomach, hardly any activity is left. At all events, excretion of thiamine in the urine does fall appreciably when cattle are placed on a bracken diet, although the animal shows no evidence of thiamine deficiency.

The causative agent of cattle bracken poisoning could be *either* (*a*) a product of thiaminase action in the rumen, e.g. a pyrimidine-cofactor compound, which is directly toxic to bone-marrow cells, *or* (*b*) a toxic chemical, in no way connected with thiaminase, present in bracken, or formed from it in the rumen, which exerts its effect directly on rapidly proliferating cells, notably of the bone-marrow.

The objectives of the present work were therefore:—

1. The elimination of one or other of the possible theories of causation.
2. An attempt to extract the poisonous principle into solution.
3. To establish a suitable biological test, so as to allow purification of the toxic agent, with the ultimate objective of its chemical isolation and identification.

Methods

Hæmatology. The methods used were those previously described by Evans *et al.*, 1954.

Thiaminase Assay. Thiaminase activity was measured manometrically by taking advantage of the fact that when thiamine is incubated with a suitable co-factor, e.g. proline, in the presence of thiaminase, the co-factor is transferred to the pyrimidine moiety of thiamine with the liberation of the thiazole part, and a hydrogen ion (H+) (see p. 19). The production of hydrogen ions is detected by displacement of CO_2 from a bicarbonate buffer, and manometric estimation of the gas evolved. The method is carried out as follows:—

Milled bracken powder (50-200 mg.), or soluble thiaminase, is placed in a Warburg flask together with 20 μ mol. of L(−)-proline and sodium bicarbonate solution is added to a final concentration of 0.1 M.; 20 μ mol. of thiamine, neutralised to pH 7.5 is added to the side-arm. The reaction is carried out at 30° C., under a gas-phase of N_2/5 per cent CO_2 mixture. Controls are included omitting proline. One unit of enzyme activity is defined as the amount of enzyme which will liberate 1 μ mol. of H+ in one hour. This method is discussed in a forthcoming publication from these laboratories, and is a modification of the suggestions of Sealock and Livermore (1944).

Experimental

1. *Attempts to produce bracken poisoning with soluble extracts*

(a) *Water*

Two yearling Welsh Mountain sheep were fistulated to the rumen, and kept in pens with a free supply of hay and water. Fresh bracken fronds (20 lb.) were collected daily, minced with ice-cold water in a Hobart power mincer. The mince was allowed to stand for two hours at 0° C., pH 8.5 being maintained by the addition of sodium hydroxide solution. The juice was pressed from the mince, through a sack in a cheese press, and centrifuged at 0° C. to remove the remaining particulate matter. The pH of the clear extract was adjusted to pH 3.0 by the addition of conc. HCl; after standing at 0° C. to allow the precipitate to flocculate, it was

collected by centrifuging in the cold. The ppt. was redissolved in 500 ml. ice-cold water, sodium hydroxide solution being added to a pH 7. This redissolved protein fraction contained the whole thiaminase content of the original extract, and was made up to a final volume of one litre. It contained 20,000 units of thiaminase activity, and was introduced via the fistula into the rumen of sheep A. The remaining acid supernatant was concentrated *in vacuo* at 20-30° C. to about 750 ml., neutralised by the addition of sodium hydroxide solution, and finally diluted to one litre. This solution was introduced via the fistula into the rumen of sheep B.

Sheep A thus received the soluble protein including thiaminase, and other acid precipitable material, from 20 lb. fresh bracken fronds, daily.

Sheep B received the non-protein soluble material from the same bracken daily.

Both animals were bled at weekly intervals for hæmatological estimations and Fig. 1 presents a summary of the results. The blood picture of a bracken-poisoned heifer is offered for comparison. Neither sheep showed any clinical symptoms, although sheep B lost weight and its general condition deteriorated. Sheep A, although having received active thiaminase extracted from approximately one ton

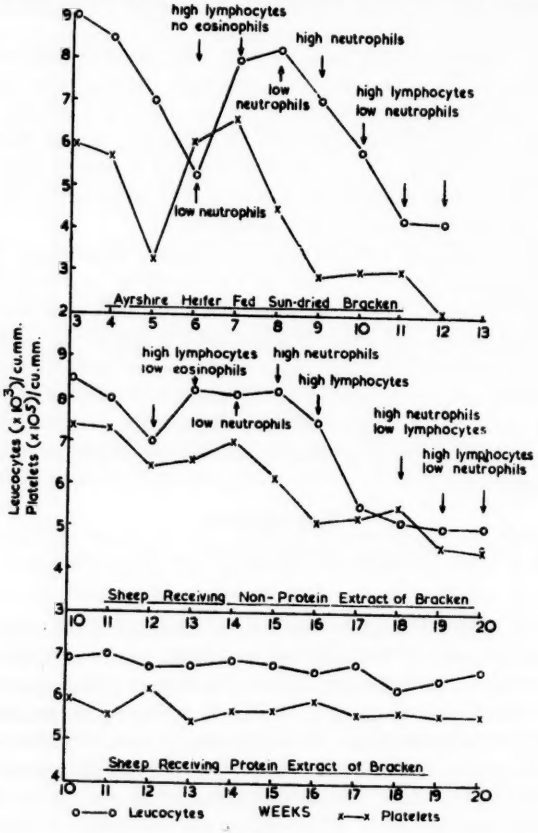


Figure 1.

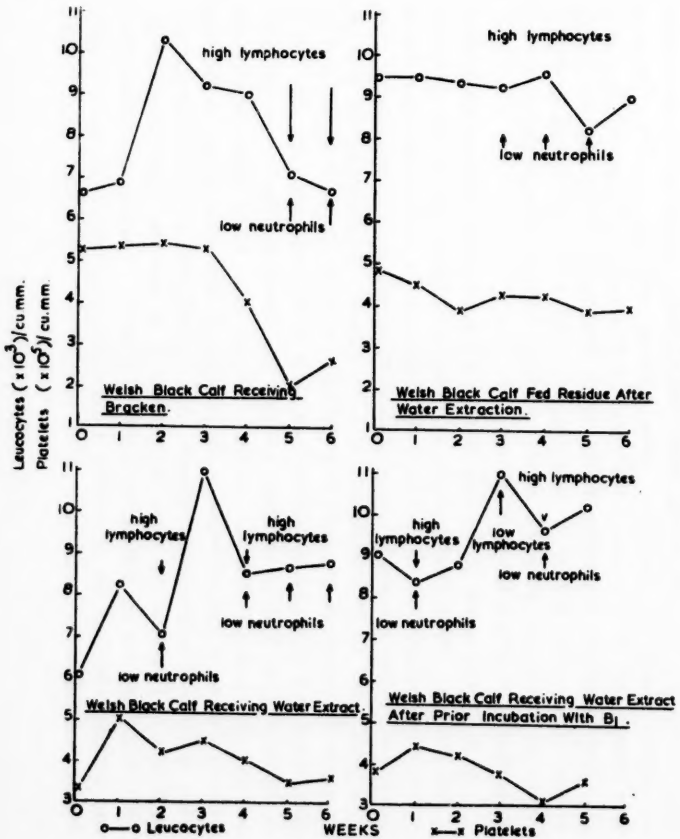


Figure 3.

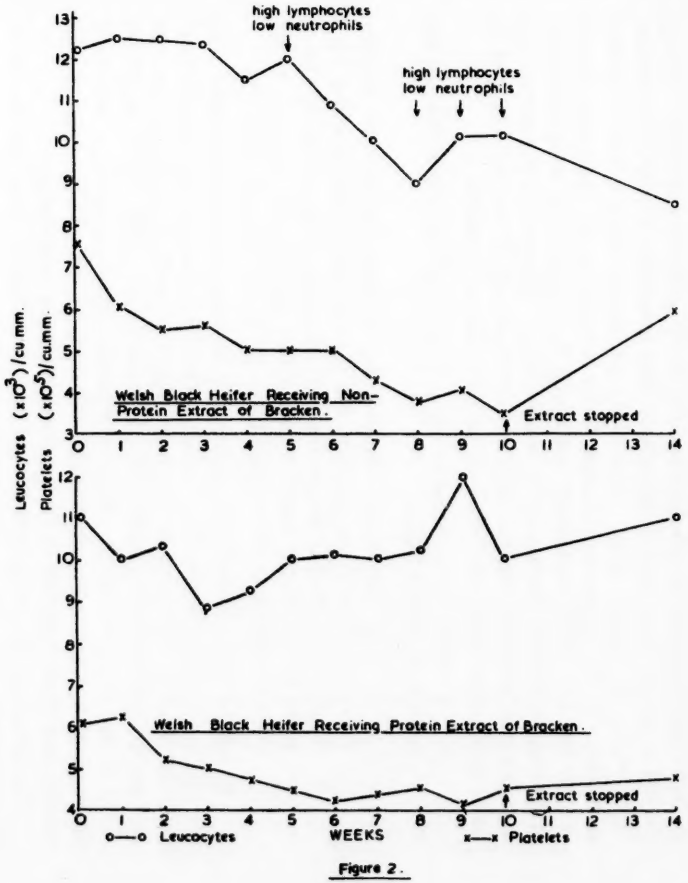


Figure 2.

of fresh bracken fronds in all (representing 3×10^6 units of thiaminase activity), showed a normal blood picture throughout, with leucocytes, platelets and erythrocytes within the normal range. The blood picture of sheep B bore a distinct resemblance to the sequence of changes found in cattle bracken poisoning, though in a far less acute form—temptingly suggesting that some of the poison is present in the small molecular fraction of the water extract. However, it is apparent that a greater part of the activity has been lost on processing, or was not extracted by water under these conditions. Two explanations seemed probable:—

i. That concentration *in vacuo* of the small molecular fraction had resulted in serious losses.

ii. That thiaminase required the co-factor peculiar to bracken. Thiaminase was in one fraction, and the co-factor would probably be in the other (i.e. small molecular fraction); the small amount of activity found might be due to the action of thiaminase and bracken co-factor on the thiamine present in the bracken plant, during mincing and extraction.

To test the first hypothesis, the experiment described above was repeated using two yearling fistulated Welsh Black heifers; the only difference was that the small molecular fraction was supplied by rumen fistula without concentration, and the acid precipitable fraction was diluted to the same volume. The results are shown in Fig. 2, and are similar to the sheep observations, with a suggestion of activity in the small molecular non-protein fraction; still, most of the toxicity of the original bracken had either been lost by these procedures or not extracted.

To investigate the second premise, 10 lb. milled, cold-air dried bracken fronds were extracted daily with ice-cold water, pH 8.5 being maintained by the addition of sodium bicarbonate solution. After standing over-night at 0° C., the juice was pressed out; it contained 1×10^5 units of thiaminase activity, and was given via rumen fistula to a yearling Welsh Black, the residue being fed to a similar animal, daily. The results of hæmatological estimations are shown in Fig. 3. Again, no appreciable activity is shown by either fraction, although there may be a suggestion of toxicity in the water extract.

At this stage, it was questioned whether a solution of thiaminase, when introduced into the rumen, might not be destroyed so rapidly that a full, prolonged action is not obtained. This should be overcome by adding thiamine during extraction, so that thiaminase action with adequate substrate concentration could take place prior to its introduction into the rumen. Accordingly, the previous experiment was repeated, adding 5 g. thiamine to each 10 lb. of milled cold-air dried bracken fronds, and incubating over-night at room temperature. The results of hæmatological findings on these animals are shown in Fig. 3; no significant activity was present in either extract. At the time when these experiments were carried out, it was believed that the products of thiaminase action on thiamine were 4-methyl-5-hydroxyethyl-thiazole, and 2-methyl-4-amino-5-hydroxymethyl-pyrimidine. This view has since been shown to be incorrect (see Thomas *et al.*, 1957) with respect to the pyrimidine moiety of thiamine. Makino and Koike (1954) had demonstrated that the pyrimidine alcohol (toxopyrimidine) can act as a pyridoxine antagonist in mice, also with certain micro-organisms, and isolated

Figure 4.

Welsh Black Calf Receiving Acetone Extracted Residue.
Welsh Black Calf Receiving Acetone Extract.
Welsh Black Calf Receiving Ether Extracted Residue.
Welsh Black Calf Receiving Ether Extract.
Leucocytes (x10³)/cu.mm.
Platelets (x10⁵)/cu.mm.
WEEKS
o—o Leucocytes x—x Platelets

Figure 5.

E

pyridoxine dependent enzymes. It was thought advisable to try its action, when introduced into the rumen of an ox.

Effect of Toxopyrimidine when introduced into the rumen of a bullock. A yearling Welsh Black was fed a hay diet. Toxopyrimidine was poured daily in solution through a rumen fistula. It was found that 2.5 g. per day was the threshold dose, above which neuromuscular disturbances occurred (hypersensitivity), 3 g. producing convulsive seizures. These symptoms were consistent with the description of induced pyridoxine deficiency in other animals, although to our knowledge it has not been produced before in the adult ruminant. The animal was given 2.0 g. toxopyrimidine per day for 35 days; apart from increased alertness, it appeared normal during this period, although it lost condition. The hæmatological results are summarised in Fig. 4, and showed no changes which could be likened to the blood picture during the development of bracken poisoning.

(b) *Organic Solvents*

Bracken, collected early in July, was dried in a stream of air at 30° C., and milled. 100 lb. of the finely milled powder was extracted three times with acetone at room temperature (using five litres of solvent per kilo of bracken). Another 100 lb. was extracted similarly with ether. The solvent phases from the extractions were separated from the bracken residues, and concentrated under reduced pressure. The almost white bracken residues were freed from solvent by low temperature drying; they still contained thiaminase, which activity remained almost unimpaired by this treatment. These dry residues were fed to two yearling Welsh Black cattle at the rate of 5 lb. per day, mixed with bran—the rest of the diet being hay.

The acetone-extracted bracken residue had an activity of 2×10^4 thiaminase units/lb.; the ether-extracted residue had 2.2×10^4 thiaminase units/lb.

The acetone and ether extracts, after removal of the solvents (and equivalent to 5 lb. dry bracken residue), were given daily to another pair of animals by rumen fistulæ; arachis oil was used to suspend the fatty, intensely green solvent extracts, and large syringes were used to introduce them into the rumen.

In addition, another fistulated animal received an ether extract of Male fern* (*Aspidium filix-mas*), 10-15 ml. being given daily via the fistula.

Figs. 5 and 6 show the results of these experiments. The animals receiving the solvent extracts showed normal values for all the blood elements. The animals receiving the bracken residues showed the characteristic hæmatological changes associated with bracken poisoning in cattle and, in fact, clinical bracken-poisoning developed.

The toxic factor was therefore not extracted by acetone or ether at room temperature; neither did a crude ether extract of male-fern (another pteridophyte) contain a factor capable of depressing bone-marrow activity.

2. *Attempts to distinguish between thiaminase and a second toxic factor, as the causative agent of bovine bracken poisoning*

The experiments already described, while suggesting that the poison may be distinct from bracken thiaminase, have not offered convincing proof that this is so.

* This was the crude ether extract of Male Fern, containing up to 5 per cent Arachis Oil, as used commercially for the preparation of the anthelmintic fraction in Danistol. It was kindly supplied by the Crown Chemical Co. Ltd., Stair, Lamberhurst, Kent.

To produce more conclusive evidence, it seemed worthwhile to study the effect of thiaminase derived from sources other than bracken, on the ruminant. These will be described.

(a) *The effect of bacterial thiaminases on cattle*

Three species of thiaminase-producing bacteria have been isolated in Japan (Fujita, 1954). *Bacillus thiaminolyticus*, Matsukawa et Misawa (BMM) is an ærobic spore-former, and *Clostridium thiaminolyticum*, Kimura et Liao (CKL) an anærobic spore-forming organism; both bacteria produce thiaminase which are broadly similar to the enzyme found in bracken, at least in their dependence on the same co-factors (Thiaminase Type I). *Bacillus aneurinolyticus* Kimura et Aoyama (BKA) the third ærobic sporer isolated, seems to contain a thiaminase which is active in the cleavage of thiamine without it being necessary to add a co-factor; a similar thiaminase has also been found in a fungus, *Trichosporon aneurinolyticum* Yonezawa et Aoki (1956) (Thiaminase Type II).

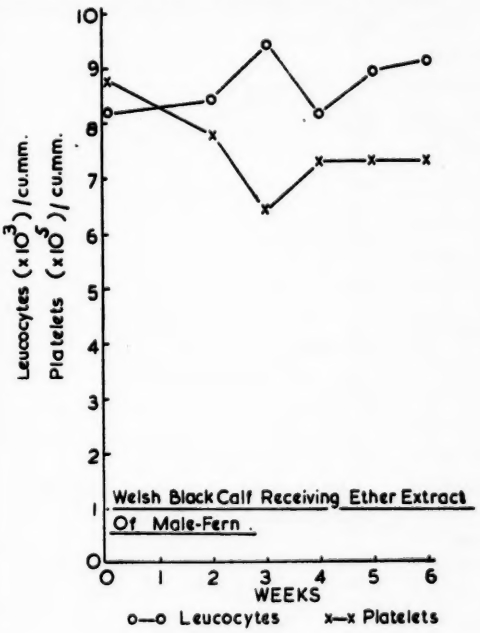


Figure 6.

The production of thiaminase by bacteria of the intestinal tract (e.g. BMM) has received considerable attention by the Japanese workers as an ætiological factor in beri-beri. "Thiaminase disease" may be induced in humans who have a disposition for it, by oral administration of a few mgms. of spun cells of BMM organism daily (Matsukawa, Chang, Misawa, Fujimiya, Kobayashi, Horikawa and Takato, 1954, 1956). Polyneuritic symptoms due to thiamine deficiency have also been produced in hens, through oral administration of BMM cultures (Matsukawa, Chang, Fujimiya and Misawa, 1955), whilst Shintani (1956) diagnosed BKA infection of the intestinal tract as the causative agent of a naturally occurring disease amongst poultry in Japan, with symptoms of motor paralysis of the extremities, essentially an avitaminosis B_1. Hamada (1956) administered BMM cultures orally to sheep, rabbits, hens, albino rats, guinea pigs and cats; the tendency to become the carrier of BMM was found to increase in this order of animals—i.e. cats developing thiaminase disease symptoms easily, whilst the organism could not be established in the alimentary tract of sheep, which remained unaffected.

It appeared feasible that if thiaminase was the causative agent of bovine bracken

poisoning, then it might be possible to reproduce the disease by administering cultures of these organisms to cattle.

*B. thiaminolyticus** (BMM) was grown in a medium containing 1 per cent nutrient broth and 0.5 per cent. glycerol, with aeration, at 37° C. for four days. A Welsh Black bullock was fed hay, and ten litres of this heavy culture was introduced daily into the rumen by fistula. After three weeks, the dose was increased to 20 litres of a well-grown culture, given in two ten-litre lots, night and morning, for a further three weeks. The results of weekly hæmatological examination of the blood are shown in Fig. 7. No significant changes were apparent; the animal thrived on this treatment, and remained healthy.

*Cl. thiaminolyticum** was grown in a glycerol-nutrient broth medium anærobically, at 37° C. for three days. A daily ten-litre culture of this organism was introduced by fistula into the rumen of a Welsh Black bullock for a month. The hæmatological results are shown in Fig. 7; again, no significant blood changes

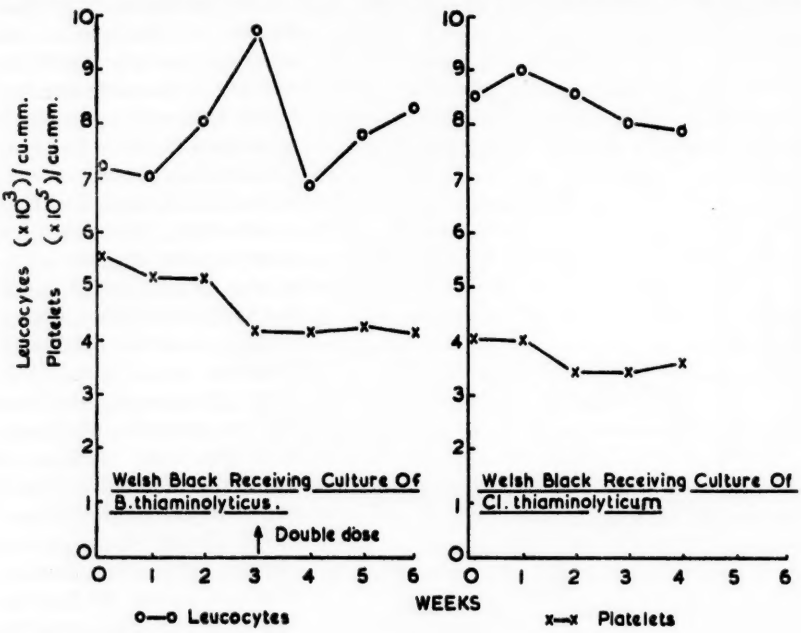


Figure 7

were apparent, and the animal remained healthy. Both cultures prior to their introduction into the rumen possessed powerful thiaminase activity (1 ml. of our BMM culture decomposed 5-20 μ g. thiamine per hour at 37° C. and pH 6.5; 1 ml. of the CKL culture decomposed 10-50 g. thiamine under similar conditions). These are comparable in strength to those reported by the Japanese workers, yet

* We are grateful to Professor R. Kimura, Microbiological Institute, Faculty of Medicine, Kyoto University, Kyoto, Japan, for sending us pure cultures of these organisms.

the enzyme had no deleterous effect on the cattle. It appeared that neither of these organisms could be established in the alimentary tract of cattle.

(b) *The effect of feeding* Equisetum arvense *to cattle*

Equisetum arvense is another pteridophyte which contains thiaminase (Evans, Jones and Evans, 1950, 1952). It also produces a condition in horses similar to bracken staggers, i.e. an avitaminosis B_1, which responds to thiamine therapy (Forenbacher, 1951, 1952). In cattle, the most marked effect of feeding *Equisetum* spp. recorded in the literature (c.f. Garner, Veterinary Toxicology, 1957, Baillière, Tindall and Cox, London) is a rapid fall in milk yield, associated with a loss of condition, and scouring; no symptoms analogous to cattle bracken poisoning has ever been attributed to *Equisetum* spp. Karrer and Eugster (1948, 1949, 1953) isolated a crystalline alkaloid, pulustrine, and dimethylsulphone from *E. palustre*, and have shown the former to cause respiratory paralysis in mice.

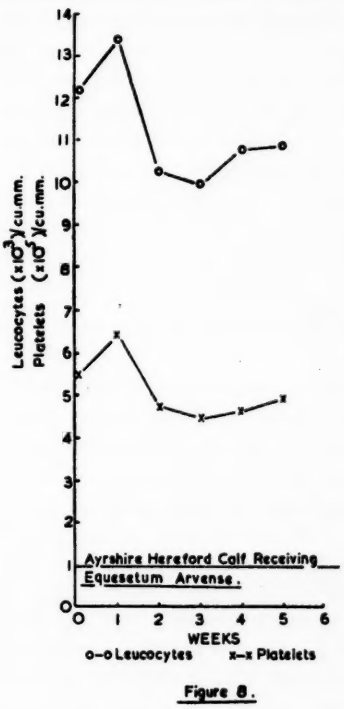


Figure 8.

It seemed probable that if thiaminase is involved in bovine bracken poisoning the condition should be equally well reproduced by feeding *E. arvense*.

A six months old heifer (Hereford-Ayrshire cross) was fed a diet composed of 50-75 per cent low temperature dried green *E. arvense* (thiaminase activity 1×10^4 units/lb.) and hay for 40 days. No untoward symptoms developed, apart from initial slight scouring; there was, however, a marked loss in condition. The animal was bled weekly for hæmatological examination, the results being shown in Fig. 8; they indicate nothing abnormal, even after 40 days.

We can definitely state that although *Equisetum arvense* possesses a thiaminase comparable to bracken, yet it does not produce the symptoms of bracken poisoning in cattle; this result is also convincing evidence that thiaminase is not the causative agent in this disease.

3. *The effect of steaming bracken on its toxicity to cattle, and the state of the rumen microflora on feeding both a steamed and unsteamed bracken/hay diet*

Evans *et al.* (1954) had reported that steaming bracken hay for an hour rendered it inocuous to cattle; yet, these workers found that when two tons fresh green bracken was put through a revolving drum grass-drier (Weldall Assembly Machine) and baled, the product still had a definite residual toxicity to cattle. It also contained active thiaminase, as determined by rat experiments. Naftalin and

Cushnie (1956) reported that fresh green bracken is still poisonous to calves after steaming for one hour.

It is our experience that fresh green bracken is considerably more potent than bracken hay. The apparent discrepancy between these findings might be explained if it is assumed that steaming the already less toxic bracken hay reduced its toxicity below a threshold level, while steaming the highly active fresh green bracken still leaves sufficient activity to produce poisoning.

To test this explanation, fresh green bracken was dried in an air stream at 30° C. and milled. This treatment is intermediate between using fresh bracken and the harshly treated, sun-dried bracken hay. The cold-air dried milled bracken was fed to one Welsh Black bullock at the rate of 5 lb. per day supplemented with hay. A similar animal was fed the same amount of milled bracken which had been autoclaved for 30 minutes at 15 lb./sq. in., and hay. After this treatment, the bracken still possessed about 2 per cent of its original thiaminase activity. The hæmatological results are presented in Fig. 9.

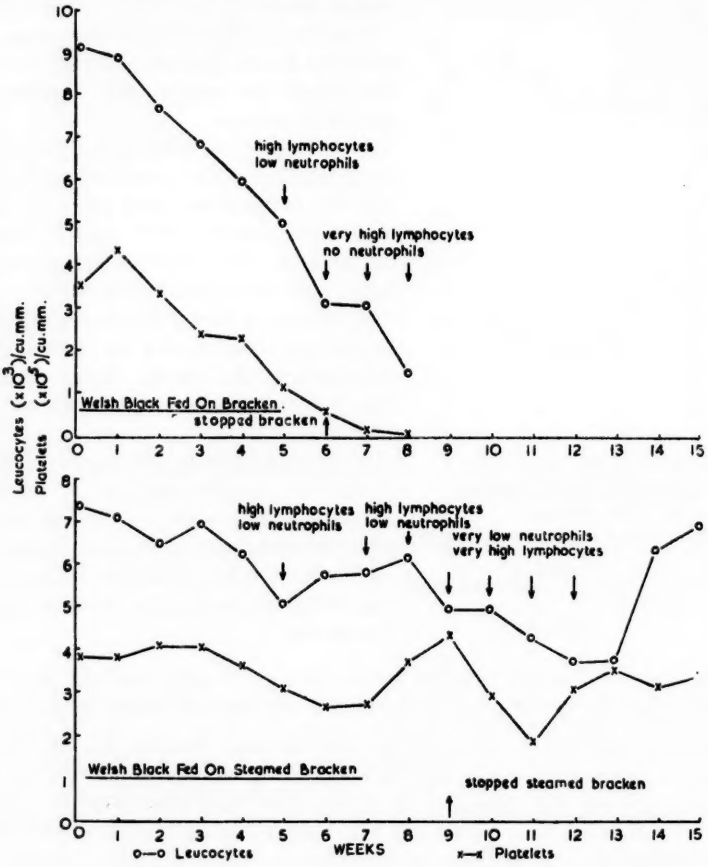


Figure 9.

The animal receiving unheated bracken showed the usual blood changes and died of bracken poisoning. The second animal, receiving the autoclaved bracken, showed changes in the blood elements simulating bracken poisoning, but they were much less marked, and clinical bracken poisoning did not develop. It seems apparent that steaming does partially inactivate the poison, but that 30 minutes at 15 lb. per square inch is insufficient to destroy it completely in fresh bracken, but does so in bracken hay. During the course of the above experiment, samples of rumen contents were taken from both animals, via a rumen fistula, at weekly intervals. These were kindly examined by Dr. C. A. E. Briggs, of the Microbiology Section, N.I.R.D., Shinfield, Reading, and counts made of the major bacterial varieties. These are presented in Table I. They show a general trend towards increased coliform counts, decreased lactobacilli counts and slightly diminished streptococcal counts. It is interesting to note that coliforms usually synthesize thiamine, while most lactobacilli and streptococci require it. The microfloral picture was similar in both animals; this can be explained, since the steamed bracken still possessed about 2 per cent of the thiaminase activity of the original air-dried bracken—enough to account for the destruction of most of the free extracellular thiamine in the rumen liquor.

Bracken thiaminase is an enzyme which *in situ* in the plant, before purification, shows a marked resistance to inactivation by heat, compared to most enzymes; it is therefore difficult to decide whether the heat treatment of bracken partially removes its toxicity to cattle by virtue of thiaminase destruction, or by the inactivation of another toxic chemical.

4. *Attempts to establish a suitable biological test to permit purification of the poison*

Efforts to extract and concentrate the "bovine bracken poison" have been greatly hampered by the severe technical difficulties of processing material on a sufficient scale to reproduce the disease in cattle. In order to ensure more rapid progress, it seemed essential that a smaller test-animal should be found.

Jones (1952) and Thomas (1954) had shown that rats fed a complete diet to which bracken powder had been added, showed a depression of bone-marrow nucleic acids and alkaline phosphatase, *even when adequate thiamine* was supplied to these animals parenterally. The depression was not acute, and no external symptoms developed, but it did seem to indicate that rat bone-marrow was affected by feeding a bracken diet even in the absence of avitaminosis B_1. It was decided, therefore, to make further intensive attempts to reproduce the "bovine bracken poisoning" syndrome in the rat. These experiments did not produce *acute* hæmatological symptoms, but with certain bracken fractions there was a transient depression of the leucocytic and thrombocytic elements in the blood. They are presented summary form only.

Hooded Wistar rats of 80-90 g. weight, housed in glass metabolism cages, fitted with a wire-screen floor and supplied with hooded feeding dishes, were used. *All* animals were supplied with adequate thiamine, either orally or by subcutaneous injection, so that there was no possibility of thiamine deficiency masking any other effect of bracken.

The groups and results were as follows:—

(i) Rats were fed *ad. lib.* on a diet comprising 50 per cent milled, low-temperature dried bracken fronds, the other 50 per cent of the diet being either powdered rat cake (Lever Bros.) or a casein semi-synthetic diet, mixing the plant powder and diet to a mash with water. The animals showed a reduced weight gain compared with those on cubed or semi-synthetic diet alone, and after about 20 days a decrease in circulating platelets and frequently depressed white cells and reticulocytes were observed. These effects were similar, irrespective of whether the semi-synthetic diet contained no thiamine, or a high concentration of this vitamin (100 mg. per kilo of diet).

(ii) Rats fed similarly, except that the bracken had been autoclaved, showed improved weight gains, and no substantial variation in the blood elements occurred.

(iii) Rats fed as in group (ii), but with the addition of a very active purified thiaminase solution (500 units/day) showed very similar weight gains and blood picture constancy as group (ii).

(iv) Finely milled, low-temperature dried bracken fronds were extracted twice at room temperature with acetone, and the bracken residue (which is still capable of producing bracken poisoning in cattle) incorporated with an equal amount of semi-synthetic died. The mixture was fed *ad. lib.* to rats. Again, these animals showed a depressed weight gain, and after about 20 days the platelet count was depressed. Prolonged feeding on this diet produced no external symptoms other than general unthriftiness.

(v) Experiment (iv) was repeated, using similar bracken but after it had been *exhaustively* extracted with acetone in a large Soxhlet apparatus. The weight gains were now increased, and all blood elements remained normal.

(vi) Experiment (i) and (iv) were repeated, but supplying the diet dry, in cube form, rather than as a wet mash. The results were similar.

(vii) Milled, cold-air dried bracken fronds were extracted with water at pH 8. The aqueous extract was concentrated *in vacuo* and given daily to each rat, mixed with semi-synthetic diet, at the rate of concentrated water extract equivalent to 50 g. bracken powder. Weight gains were very good and no blood changes could be detected.

(viii) Experiment (vii) was repeated, but with the difference that the bracken was extracted at pH 3. Again weight gains were good, and there were no blood changes.

(ix) Milled, cold-air dried bracken was placed in a large flask, and steam blown through. The distillate was collected. From each kilo of bracken powder, one litre of distillate was obtained, and this was added to the semi-synthetic diet at a rate of 20 ml. per day per rat. Weight gains were normal, and no blood changes could be observed.

(x) Milled cold-air dried bracken (2 kilos) was extracted once with acetone, at room temperature. The acetone-extracted bracken powder was then re-extracted, five times, with a volume of ethanol sufficient to cover the powder. The ethanol extract was bulked and the solvent removed *in vacuo*, and the ethanol soluble residue then mixed with two kilos of powdered rat cake. This was fed *ad. lib.* to rats. After about three weeks, a slight bloody nasal discharge was evident, and there was a certain amount of bleeding from the ears of these animals. Flecks of mucous were noticed occasionally in the fæces. Weight gains were less than controls, and a blood count after three weeks showed depressed leucocyte and platelet numbers; these subsequently increased and returned to within the normal range.

The most significant finding in these series of rat experiments, is that ethanol extracts a factor with an effect on the rat simulating, although never in such an acute form, the characteristic hæmatological picture in cattle bracken poisoning. It was clearly imperative that this experiment should now be scaled up for trial with cattle.

5. *The production of bracken poisoning in cattle by feeding an ethanol soluble fraction of bracken*

The low-temperature dried, green bracken powder, of potency similar to that used in experiments described in Section 1 (b), organic solvents (p. 7), was first extracted with acetone at room temperature. This procedure serves to dehydrate the powder as well as removing pigment and other inactive material, without impairing the toxicity of the bracken residue to cattle (see p. 8). Boiling ethanol was then poured over the powder (using five litres of ethanol per kilo of bracken residue), the mixture boiled again for a few minutes, and filtered hot, with suction, through several layers of cheese-cloth in large Buchner funnels. This boiling ethanol extraction was repeated once for the first month of the experiment, but a third extraction was introduced after this period. The ethanol extracts were bulked, sucked through a large Berkefeld candle to remove *all* particulate matter, and the solvent distilled at 20-30° C. under reduced pressure. The ethanol soluble concentrate from two kilos of bracken powder was then mixed with bran, all traces of solvent removed in a current of warm air, and fed daily to a six months old Welsh Black bullock (G 7). The bracken residue (two kilos) left over after this ethanol extraction was similarly freed from solvent, mixed with bran, and fed daily to another six months old Welsh Black bullock (G 9). Both animals received chaffed hay and water *ad. lib.* The animals were bled periodically, and the hæmatological results are shown in Fig. 10.

Both animals developed all the characteristic symptoms of bracken poisoning, and, in fact, died of the disease within two days of each other. The diagnosis was confirmed by post-mortem examination. It would seem that the activity was distributed about equally between the two fractions. Comparison of the time required to produce clinical bracken poisoning by either of these fractions (Fig. 10, nine weeks) with the corresponding period using a similar amount of acetone or ether

extracted bracken residues (Fig. 5, five weeks) is in accord with this apparent halving of the dose.

This result is a decisive step in the search for the toxic principle in bracken responsible for cattle bracken poisoning; it is not thiaminase, but another substance which is soluble to some extent in hot ethanol.

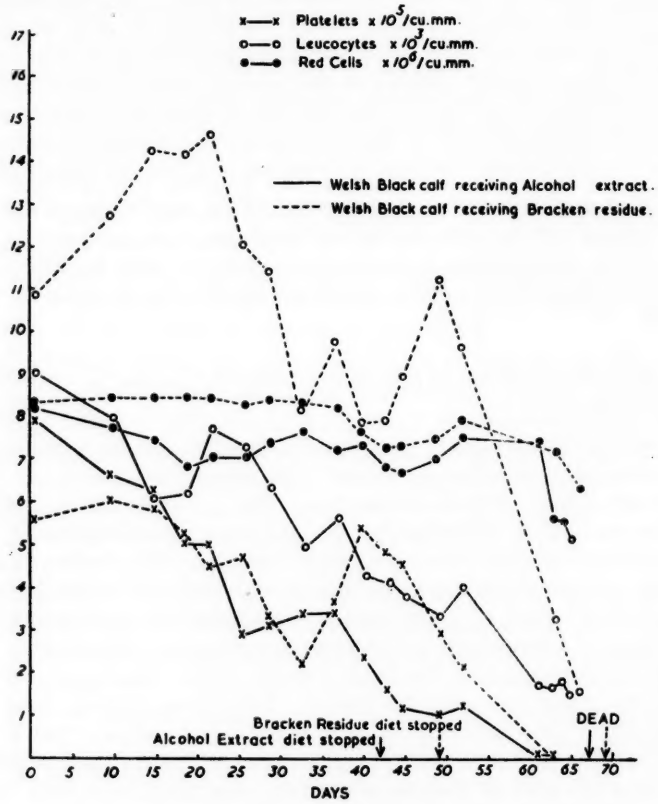


Figure 10.

Discussion

The consequences of the inclusion of bracken in the feed of animals are as follows:—

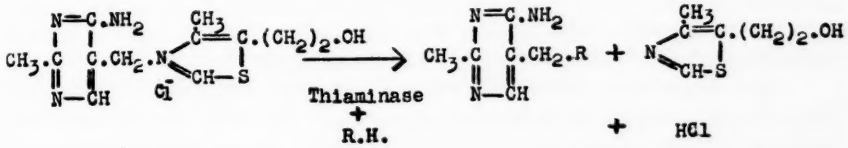
(a) In simple-stomached animals an avitaminosis B_1 develops; a classical example of this is " bracken staggers " in the horse.

(b) In the ruminant, there is no avitaminosis B_1, but a bone-marrow aplasia leading to a severe leucopenia and thrombocytopenia, makes its appearance. This is the characteristic lesion in cattle bracken poisoning.

Two types of the enzyme thiaminase have been discovered. Thiaminase I occurs in certain micro-organisms, some plants (notably the pteridophytes), clams, and the viscera of fish of the carp family. This enzyme activates thiamine for a nucleophilic displacement of the thiazole moiety by a suitable co-factor; the particular compound which couples with the pyrimidine part depends on which co-factor happens to be present. Thiaminase II, present in *B. aneurinolyticus* requires no co-factor for its activity, the products being the pyrimidine alcohol (toxopyrimidine) and the thiazole moiety. The inactivation of thiamine is represented as follows:—

In Thiaminase I, the cofactor or nucleophilic agent (R.H.) may be any one of a large variety of compounds of the general type:—aromatic amines, e.g. m-aminobenzoic acid or aniline; heterocyclic amines, e.g. pyridine or proline; sulphur containing amino-acids, e.g. cysteine or hypotaurine.

For Thiaminase II, R = OH.



In bracken, we have detected the presence of proline (Watkins, Thomas and Evans, 1953) and pyridine (Chamberlain, Borginon, Thomas and Evans, 1958), both of which are capable co-factors in this reaction; there are other as yet unidentified co-factors present in this plant.

Clam tissue, acting on thiamine gives rise to a compound "icthiamine", the constitution of which has recently been elucidated by Kupstas and Hennessy (1957) as 2-methyl-4-amino-5-(2-aminoethanesulphonyl)-methylpyrimidine; hypotaurine is the naturally occurring nucleophilic agent in this case.

It will be appreciated that, in addition to rendering a diet thiamine-free, this enzyme (Thiaminase I) also produces an unusual pyrimidine derivative. Certain synthetic pyrimidines are antimetabolites of nucleic acid metabolism (Wright, 1951); toxopyrimidine itself is a potent antagonist of pyridoxine (Makino and Koike, 1954).

Thiaminase was the only known nutritionally harmful agent present in bracken, and it is not thought to have any action other than that depicted above. It is easy to understand how this enzyme produces an avitaminosis B_1 in simple stomached animals, and even why it does not do so in ruminants (p. 2); how it could also cause bone-marrow lesions of cattle bracken poisoning has been difficult to visualise, unless it be through some antimetabolite or allergic mechanism. If it can be demonstrated that thiaminase is not able to produce the cattle disease, then it must follow that there is another toxic factor(s) present in bracken whose action is masked in simple stomached animals through the prior onset of avitaminosis B_1, but revealed in the ruminant since the vitamin deficiency does not appear.

The failure of purified bracken thiaminase, the various bacterial thiaminases, and especially *Equisetum arvense* whole plant (another thiaminase containing pteridophyte), when fed to cattle, to elicit the bovine syndrome, leads us to the conclusion that this enzyme is not the causative agent in the cattle disease. The only obvious loop-hole in this argument is the possibility that bracken might contain

a specific co-factor (removed during the preparation of the enzyme from bracken and not present in the other sources) which attaches itself on to the pyrimidine moiety of thiamine to produce a unique, toxic, pyrimidine complex. Toxopyrimidine, at any rate, is incapable of inducing the disease, although in sufficient dose it has other effects in cattle.

If there is a bone-marrow poison in bracken, one might expect that other animal species would show analogous lesions to cattle, provided the effect of thiaminase was nullified through thiamine supplementation. On the other hand, cattle may be peculiarly sensitive to this second factor—a situation similar in some ways to Tri-Chloroethylene-Extracted-Soybean-Oil-Meal (TCESOM) poisoning, without, of course, implying that they are due to the same causative agent. TCESOM poisoning of cattle is an aplastic anæmia (Pritchard, Rehfeld, Mizuno, Sautter and Schultze, 1956), with almost identical end lesions to cattle bracken poisoning. This syndrome was also produced in the horse, but less readily in sheep, whilst rats, guinea pigs, hamsters, rabbits, mice, dogs and poultry although showing general unthriftiness, did not show the characteristic hæmatological changes.

When Evans *et al.* (1949, 1951) were investigating experimentally produced " bracken staggers " in the horse, they were mainly concerned with the symptoms resulting from the induced avitaminosis B_1; although a leucocytosis was present, no platelet counts were done. Later, these workers encountered field cases and recorded greatly depressed thrombocyte counts in the two animals affected. Its significance was not apparent at the time, but it now seems possible that the horses were beginning to show the hæmatological changes caused by the second toxic agent in bracken.

Incontrovertible evidence for the presence of a second poison was finally obtained through its extraction into an organic solvent, and the reproduction of the cattle disease by this fraction. Previous attempts to accomplish this had been unsuccessful (Shearer, 1945); our own preliminary work in this direction also gave negative results, mainly because of lack of knowledge of the solubility and stability of this second factor. It was at this stage that a renewed attempt was made to use the rat as the test animal, to circumvent the laborious procedure of having to try each fraction in the ox. This led to the use of hot ethanol as the solvent of choice.

Aqueous deproteinised extracts of bracken, prepared under a variety of conditions, contained a small fraction of the total activity of the original bracken; moreover, the bracken residue had also lost most of its potency. Neither fraction produced clinical bracken poisoning in cattle. It appears that the factor is unstable under these conditions—either through enzymatic attack or chemical combination with auto-oxidizable components of the plant.

Neither acetone nor ether extracted the bovine poison at room temperature; the bracken residues retained unimpaired their toxicity for cattle. The thiaminase content of these residues was almost identical with that of the original bracken.

When the active bracken powder was extracted with boiling ethanol, enough of the bovine poison passed into solution so that on removal of the solvent, the *ethanol soluble* fraction caused clinical bracken poisoning in an ox. Even after three, consecutive, hot ethanol extractions (using five litres of ethanol to one kilo of bracken powder per extraction), there remained appreciable activity still in the

bracken residue—enough also to cause clinical manifestations of bracken poisoning in a bullock. It appeared from our toxicity experiments that we were extracting about half of the bovine poison originally present in the bracken into ethanol; the quantities involved precluded us from attempting exhaustive extraction.

This result proves unquestionably that thiaminase need not be present in a bracken fraction to produce the fatal disease in cattle, and also establishes beyond doubt that the bovine poison is a separate entity soluble in ethanol. The next step is the isolation of the factor(s), and this task is in hand.

Summary

1. Efforts to reproduce cattle bracken poisoning by feeding a purified bracken thiaminase preparation, or other sources of this enzyme, e.g. bacterial thiaminase, and horse-tail (*Equisetum arvense*), were unsuccessful. These results provide strong evidence that thiaminase is not the causative agent of the cattle disease.

2. Toxopyrimidine, when introduced into the rumen of an ox did not cause bracken poisoning. In sufficient dose it precipitated convulsive seizures, reminiscent of induced pyridoxine deficiency in other animals.

3. The rumen flora of a bracken-fed bullock showed a general trend towards increased coliform counts, decreased lactobacilli and slightly diminished streptococcal counts—but these tendencies were not very marked.

4. The possibility of eliciting cattle-type bracken poisoning lesions in rats has been explored. Acute agranulocytosis and thrombocytopenia, with its terminal sequelæ, were not consistently produced by feeding any of the bracken fractions at the dose levels tried. Instead, a transient leucopenia and thrombocytopenia with sometimes petechial hæmorrhages on mucous membranes developed. The animals, although supplemented with thiamine, lost weight and some died.

5. Attempts were made to reproduce cattle bracken poisoning by soluble extracts of the fern, with the following results.

(a) Water extraction of bracken gave equivocal results. Neither the deproteinised water extract or the bracken residue had much activity.

(b) Ether or acetone, at ordinary room temperature, failed to extract the poison; the bracken residues, however, remained fully active.

(c) Cattle bracken poisoning was reproduced by feeding an ethanol soluble fraction of bracken. This result eliminates the enzyme thiaminase as the causative agent in this disease, and establishes that it is due to another substance, extractable by hot ethanol.

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SOME OBSERVATIONS ON THE ANTIGENIC STRUCTURE OF ESCHERICHIA COLI ISOLATED FROM THE DOG

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THE frequency with which *Escherichia coli* is isolated from the dog in diverse pathological conditions raises the question of its possible pathogenicity for that

species, but as no clinically defined syndrome such as that of calf scours is recognised in the dog, the possible significance of its *E. coli* is difficult to assess.

An approach to the problem was made by examining the antigenic structure of strains, looking particularly for relationships to the recognised groups of *E. coli* associated with calf scours (Lovell 1937, 1951) or infantile diarrhœa (Charter and Taylor 1952, Charter 1956) and for any evidence of groups apparently specific for the dog.

Materials and Methods

Three strains of *E. coli*, RVC 2194, 2120 and 51 (0.114, B?) and their homologous sera prepared in rabbits according to the method of Vahlne (1948) were supplied by Dr. T. A. Rees, of the Royal Veterinary College, and used as a basis for the test. The first two strains had been isolated from vaginal swabs of Foxhounds with a history of poor litters and stillbirths, and from canine mastitis respectively, while RVC 51 had been isolated from the fæces of a healthy dog and found to belong to group 0.114 commonly associated with calf scours. Fresh strains isolated from the dog were tested against these three antisera by slide and tube agglutination technique. Those strains which failed to show agglutination were used to prepare antisera in rabbits, until a group of 22 sera were obtained all of which were different as shown by cross agglutination tests and agglutinin absorption techniques. These antisera were then used as test sera for examining subsequent strains isolated.

All strains of *E. coli* examined serologically belonged to the Fæcal Type I group, as defined by Wilson (1935), and therefore showed positive reactions to the indole and methyl-red tests, a negative reaction to the Vosges-Proskauer test, and the absence of growth in Koser's citrate medium. Many of these were isolated from lung, spleen and heart-blood at post-mortem examination, and the remainder from skin and ear abcesses, tonsils, uterus and vagina. Tests were made for both O and K antigens as defined by Kaufmann (1947).

Results

Of the 22 strains used for the preparation of antisera, three were those supplied by Dr. Rees and one was identified as 0.101. The 18 remaining strains were tested by Dr. Joan Taylor against 29 sera prepared against strains of *E. coli* of infantile diarrhœa type; none showed any relationship with 0.9, 0.10, 0.20, 0.25, 0.26, 0.28, 0.35, 0.40, 0.44, 0.55, 0.78, 0.86, 0.103, 0.111, 0.112, 0.114, 0.119, 0.124, 0.125, 0.126, 0.127, 0.128, E.1073 (1), E.1385 (3), E.1225 (2), E.2581, E.7120, E.7148, E.68/56.

In all, 149 strains of *E. coli* were examined, the first 96 being tested against a gradually increasing number of antisera as these were accumulated, while the last 53 strains were tested against all 22 antisera.

Ten strains were agglutinated in virtue of O and K antigens; of these nine belonged to one or other of seven serotypes and one was identical with a calf strain (0.101). Four were agglutinated in virtue of their O antigen alone and ten in virtue of K alone; six of these fourteen strains showed some antigenic relationship with RVC 51 (0.114) or DCS 2176 (0.101). The results are given in Table I.

Conclusion

In the course of these limited observation, it has been possible to classify ten

canine strains only according to their O and K antigens. Of these, one only could be identified completely as belonging to a group associated with calf scours, although a limited relationship was apparent with six additional strains. No serological relationship was found between strains isolated from infantile diarrhœa and from dogs.

TABLE 1

Results of Attempts to Classify Strains of E. coli from Dogs, by Serological Methods.

No. of strains tested	No. of strains agglutinated in respect of O and K antigens	No. of strains agglutinated in respect of		No. of strains which were not agglutinated by any sera used
		O antigen alone	K antigen alone	
149	10	4	10	125

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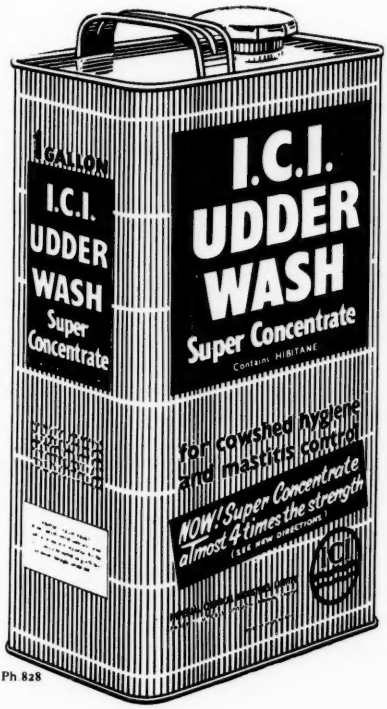

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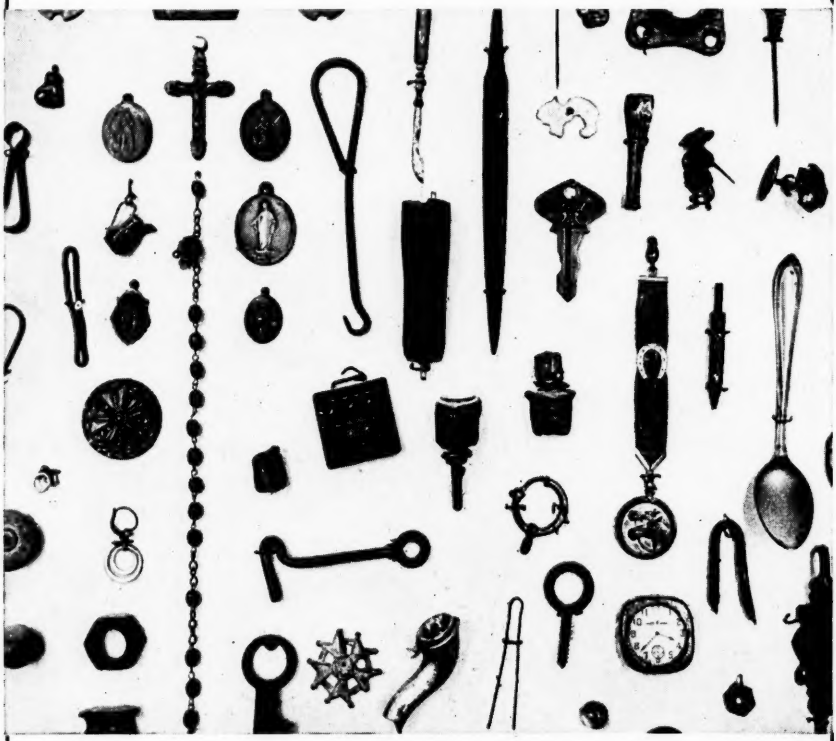

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